HEPATITIS B VACCINE (rDNA) BRP (Method A, Merck & Co – thiomersal free) batch 2

Hepatitis B vaccine (rDNA) BRP (Method A, Merck & Co – thiomersal free) batch 2 is for use in the context of the in vitro potency assay of yeast-derived hepatitis B vaccines, which can be assessed according to the Ph. Eur. General Chapter 2.7.15, using method A. The details of method A are provided below. Hepatitis B vaccines derived from other sources cannot be assessed by this method and with this BRP. The antigen content assay must be preceded by a product specific desorption process which is included in method A.

The BRP consists of liquid, adsorbed, yeast-derived rDNA hepatitis B surface antigen (HBsAg). The BRP is presented in vials with an assigned HBsAg content of

16.6 µg/ml

STORAGE

Keep vials unopened at +2 - +8°C. Do not freeze because this will destroy the adjuvant.

METHOD A

Mix all vaccines and vaccine dilutions well before further use to avoid depositing of the adsorbed antigen.

Reagents used for sample pre-treatment:

- Diethanolamine (DEA): Kodak Art. 1131275 or equivalent (can be stored at room temperature in an amber bottle indefinitely; product may thicken or crystallise at room temperature). Warm to 30°C to liquify for ease of pipetting.
- Triton X-100 (Fisher Scientific Art. BP-151-100 or equivalent); store at room temperature.
PLEASE NOTE THE FOLLOWING SAFETY PRECAUTIONS.
Handle diethanolamine and Triton X-100 with care as they may cause irritation of the skin, eyes and mucous membranes.

Reagents from the Auszyme ELISA kit:
- conjugate-solution: Auszyme monoclonal conjugate
- plastic beads coated with anti-HBsAg monoclonal antibodies (Anti-HBs monoclonal coated beads)
- positive control
- negative control
- OPD-tablets
- diluent for OPD

PLEASE NOTE THE SAFETY PRECAUTIONS MENTIONED IN THE KIT LEAFLET.

Other reagents:
- NaCl: Merck Art. 6404 or equivalent
- Na₂B₄O₇
- Na₂HPO₄: Merck Art. 6586 or equivalent
- NaH₂PO₄
- H₂SO₄ 95-97%: Merck Art. 731 or equivalent (e.g. from kit)
- glass distilled water
- Bovine Serum Albumin (BSA): Sigma Art. 4503 or equivalent (to be kept in refrigerator).

PREPARATION OF SOLUTIONS:
Sample pre-treatment solution:
- 2.5% Diethanolamine, 0.2% Triton X-100 in PBS-solution (see hereafter)
Other solutions:

- **PBS-solution**: (Phosphate Buffered Saline): Dissolve and add up to 1000 ml distilled water: 8.850 g NaCl, 0.674 g NaH$_2$PO$_4$ and 0.200 g Na$_2$HPO$_4$. Mix well. Adjust to pH 7.2 with HCl or NaOH. Store at room temperature.

- **Thimerosal 1%**: Dissolve and add up to 1000 ml distilled water: 14 g Na$_2$B$_4$O$_7$, 10 g Thimerosal.

- **Vaccine diluent**: PBS + 1% BSA + 0.01% thimerosal: add to 10 g BSA, 800 ml of PBS solution and 10 ml of thimerosal 1%. Mix well and add up to 1000 ml. Keep in refrigerator.

- **OPD-substrate solution**: dissolve one tablet of OPD per 5 ml of diluent. Prepare freshly, 5 to 10 minutes before use.

- **Stop reagent**: 1N H$_2$SO$_4$. Store in brown glass bottle at room temperature or from the kit.

**VACCINE DILUTIONS:**

**Pre-treatment procedure:**

- a volume of 0.1 ml of sample pre-treatment solution is added and well-mixed to 0.1 ml of each undiluted vaccine (dilution 1/2)

- after 30-35 minutes at room temperature (20-28°C) the vaccine dilutions are immediately prepared.

**Vaccine dilutions:**

Three identical dilution series are prepared for each assay. Each dilution is tested only once.

The following dilution scheme has been tested and found suitable. Adaptations to local conditions or to observed optical densities may be needed. Observed optical densities should fall within the linear part of the dilution-OD curve. It is advised to perform preliminary assays to determine optimal conditions.

**Pre-dilutions: (prepare in triplicate)**

<table>
<thead>
<tr>
<th>Preparation of Pre-dilutions</th>
<th>Final Vaccine Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>A) 50 µl vaccine + 950 µl vaccine diluent</td>
<td>1/40</td>
</tr>
<tr>
<td>B) 200 µl A + 1800 µl vaccine diluent</td>
<td>1/400</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Preparation of Dilution</th>
<th>Final Vaccine Dilution</th>
</tr>
</thead>
</table>
1) 25 µl B + 975 µl vaccine diluent 1/16000  
2) 50 µl B + 950 µl vaccine diluent 1/8000  
3) 100 µl B + 900 µl vaccine diluent 1/4000  
4) 200 µl B + 800 µl vaccine diluent 1/2000  
5) 400 µl B + 600 µl vaccine diluent 1/1000  

TESTING AND MEASURING
Please use the same lot of Auszyme® kit within one independent assay. Please read the leaflet which comes with the kit carefully and comply with its specifications.
1. 200 µl of each working dilution is transferred to the wells of the microtitre plate (N.B.: use a new tip for each transfer). Also transfer 200 µl of negative and positive control to three and two wells respectively.
2. Add 50 µl conjugate solution to each well in use. Mix by tapping.
3. Add a bead to each well in use, cover the plates with paper and tap to mix.
4. Incubate the plates for 3 hours at 40 °C in a water bath. An incubation period of 12 to 20 hours at room temperature has also been used with satisfactory results. Please choose the incubation conditions routinely used in your lab.
5. Prepare the OPD-substrate 5 to 10 minutes before the three hour period has ended.
6. Remove the plates from the water bath, remove paper covers, remove supernatant and wash three times with distilled water.
7. Transfer beads to tubes.
8. Add 300 µl of the freshly prepared OPD-substrate to each bead containing tube and to one additional beadless tube (substrate blank).
9. Cover tubes with paper and keep in the dark for 30 minutes at room temperature.
10. After 30 minutes, remove tubes from dark and add 1 ml stop reagent to each tube.
11. Measure ODs at 492 nm. Measurement is valid if: OD positive control - OD negative control N = not smaller than 0.400
12. Check substrate blank visually for abnormal colouring.

CAUTION
Hepatitis B vaccine (rDNA) BRP (method A – thiomersal free) Batch 2 is not appropriate for administration to humans and/or to animals. This preparation must be handled according to the appropriate QA system for biological testing laboratories. Please refer to the corresponding safety data sheet, which can be downloaded from the internet web site of the EDQM (http://www.edqm.eu) or delivered upon request.
LITERATURE